

-239-

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description  
 on page 5, line 8

## B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet 

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (including postal code and country)

12301 Parklawn Drive  
Rockville, Maryland 20852  
United States of America

Date of deposit

September 23, 1996

Accession Number

97727

C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet 

DNA plasmid PAI-2

## D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

## E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

For receiving Office use only

 This sheet was received with the international application

Authorized officer

For International Bureau use only

 This sheet was received by the International Bureau on:

Authorized officer

-240-

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>5</u> , line <u>14</u>		
B. IDENTIFICATION OF DEPOSIT		Further deposits are identified on an additional sheet <input type="checkbox"/>
Name of depositary institution American Type Culture Collection (ATCC)		
Address of depositary institution (including postal code and country) 12301 Parklawn Drive Rockville, Maryland 20852 United States of America		
Date of deposit September 23, 1996	Accession Number	98176
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>		
Escherichia coli, 596		
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)		
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")		
<div style="display: flex; justify-content: space-around;"> <div style="width: 45%;"> <p>For receiving Office use only</p> <p><input type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer</p> </div> <div style="width: 45%;"> <p>For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p> </div> </div>		

**What Is Claimed Is:**

1           1. An isolated nucleic acid molecule, comprising a polynucleotide  
2           having a nucleotide sequence at least 95% identical to a sequence selected from  
3           the group consisting of:

4           (a) a nucleotide sequence of an open reading frame depicted in one of  
5           Tables 1 through 4;

6           (b) a nucleotide sequence beginning with the first initiation codon  
7           encountered reading 5' to 3' in an open reading frame depicted in one of Tables 1  
8           through 4, and ending with the 3' terminal stop codon;

9           (c) a nucleotide sequence beginning with the first initiation codon  
10          encounter reading 5' to 3' in an open reading frame depicted in one of Tables 1  
11          through 4, and ending with the nucleotide preceeding the 3' terminal stop codon;

12          (d) a nucleotide sequence of (a) excluding codons for amino acids  
13          eliminated during processing of the putative protein identified in one of Tables 1  
14          through 4; or

15          (e) a nucleotide sequence that is complementary to any of the  
16          nucleotide sequences in (a), (b), (c), or (d).

1           2. An isolated nucleic acid molecule of claim 1, wherein said  
2           nucleotide sequence is 100% identical to the nucleotide sequence of an open  
3           reading frame depicted in Tables 1 through 4, or a complement thereof.

1           3. An isolated nucleic acid molecule, comprising a polynucleotide that  
2           hybridizes under stringent hybridization conditions to a nucleic acid molecule of  
3           claim 2.

1           4. An isolated nucleic acid molecule, comprising a polynucleotide that  
2           encodes the amino acid sequence of an epitope-bearing portion of an *E. coli* J96  
3           PAI protein encoded by an open reading frame depicted in one of Tables 1  
4           through 4.

1           5.     A method of making a recombinant vector, comprising inserting  
2     an isolated nucleic acid molecule of claim 1 into a vector.

1           6.     A recombinant vector produced by the method of claim 5.

1           7.     A method of making a recombinant host cell, comprising  
2     introducing a recombinant vector of claim 6 into a host cell.

1           8.     A recombinant host cell produced by the method of claim 7.

1           9.     A recombinant method for producing an *E. coli* J96 PAI  
2     polypeptide, comprising culturing a recombinant host cell of claim 8 under  
3     conditions such that said polypeptide is expressed and recovering said polypeptide.

1           10.    An isolated polypeptide of an *E. coli* J96 PAI IV or PAI V protein  
2     encoded by a polynucleotide of claim 1.

1           11.    An isolated polypeptide of an *E. coli* J96 PAI IV or PAI V protein  
2     encoded by a polynucleotide of claim 2.

1           12.    An isolated polypeptide comprising an immunogenic epitope of an  
2     *E. coli* J96 PAI IV or PAI V protein encoded for by an open reading frame  
3     depicted in one of Tables 1, 2, 3 or 4.

1           13.    A vaccine, in dosage form, comprising  
2     (a)    a pharmaceutically acceptable diluent, carrier, or excipient, and  
3     (b)    an antigen selected from the group consisting of:  
4               (i) a polypeptide having an amino acid sequence at least 95% identical to  
5     an amino acid sequence encoded by a uropathogenic *E. coli* J96 PAI IV or PAI  
6     V open reading frame depicted in Tables 1, 2, 3 or 4, and  
7               (ii) a polypeptide comprising an immunogenic epitope of an *E. coli* J96  
8     PAI IV or PAI V protein encoded for by an open reading frame depicted in one  
9     of Tables 1, 2, 3 or 4;

-243-

10 wherein said antigen is present in an amount effective to elicit protective immune  
11 responses in an animal to pathogenic *E. coli*.

1 14. An isolated antibody that binds specifically to a polypeptide of  
2 claim 10 or 11.

1 15. An antibody having binding affinity to a polypeptide according to  
2 claim 12.

1 16. A method of detecting a pathogenic *E. coli* antigen in a sample,  
2 comprising:

3 (a) contacting said sample with an antibody according to claim 14 or  
4 15 under conditions such that immunocomplexes form, and  
5 (b) detecting the presence of said antibody bound to said antigen.

1 17. A diagnostic kit comprising:  
2 (a) a first container means containing an antibody according to claim  
3 14 or 15 and  
4 (b) second container means containing a conjugate comprising a  
5 binding partner of said antibody and a label.

1 18. A hybridoma which produces an antibody according to claim 14  
2 or 15.

1 19. A method of detecting the presence of antibodies to pathogenic *E.*  
2 *coli* in a sample, comprising:  
3 (a) contacting said sample with a polypeptide according to one of  
4 claims 10, 11 or 12 under conditions such that immunocomplexes form, and  
5 (b) detecting the presence of said antibody bound to said antigen.

1 20. A kit for detecting the presence of antibodies to pathogenic *E. coli*  
2 in a sample comprising at least one container means having disposed therein a  
3 polypeptide according to one of claims 10, 11 or 12.

1           21. Computer readable medium having recorded thereon one or more  
2           nucleotide sequences depicted in SEQ ID NOs: 1 through 142, or nucleotide  
3           sequences at least 99.9% identical thereto.

1           22. Computer readable medium having recorded thereon a nucleotide  
2           sequence of at least one uropathogenic *E. coli* J96 pathogenicity island open  
3           reading frame depicted in Tables 1 through 4, or a complement thereof.

1           23. The computer readable medium of claim 21, wherein said medium  
2           is selected from the group consisting of a floppy disc, a hard disc, random access  
3           memory (RAM), read only memory (ROM), and CD-ROM.

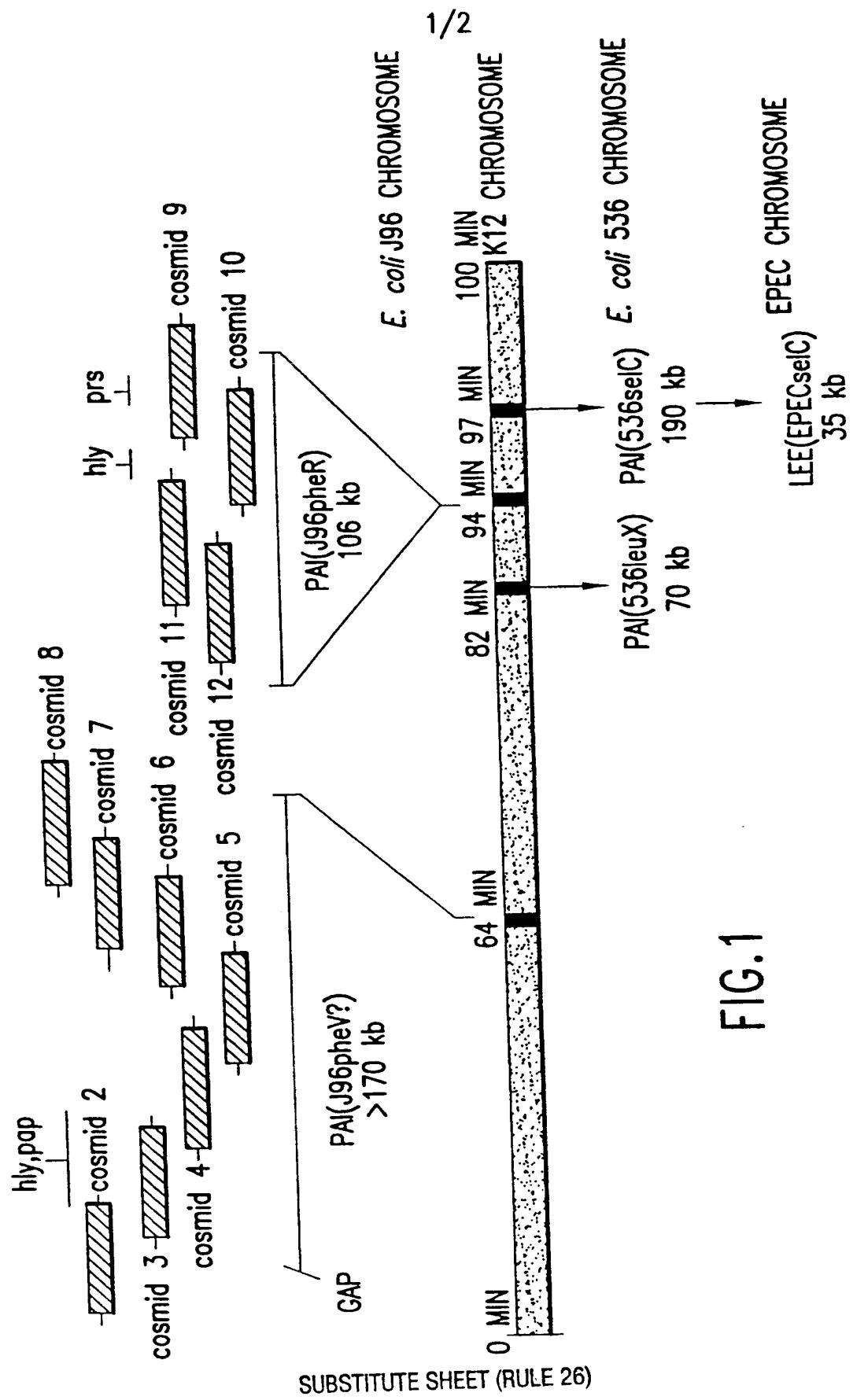
1           24. The computer readable medium of claim 22, wherein said medium  
2           is selected from the group consisting of a floppy disc, a hard disc, random access  
3           memory (RAM), read only memory (ROM), and CD-ROM.

1           25. A computer-based system for identifying fragments of  
2           uropathogenic *E. coli* J96 pathogenicity islands PAI IV and PAI V that are  
3           homologous to target nucleotide sequences, comprising:

4           a) a data storage means comprising a nucleotide sequence of  
5           SEQ ID NOs: 1 through 142, or a nucleotide sequence at least 99.9% identical  
6           thereto;

7           b) a search means for comparing a target sequence to said  
8           nucleotide sequence of said data storage means of step (a) to identify a  
9           homologous sequence, and

10           c) a retrieval means for obtaining said homologous sequence  
11           of step (b).



2/2

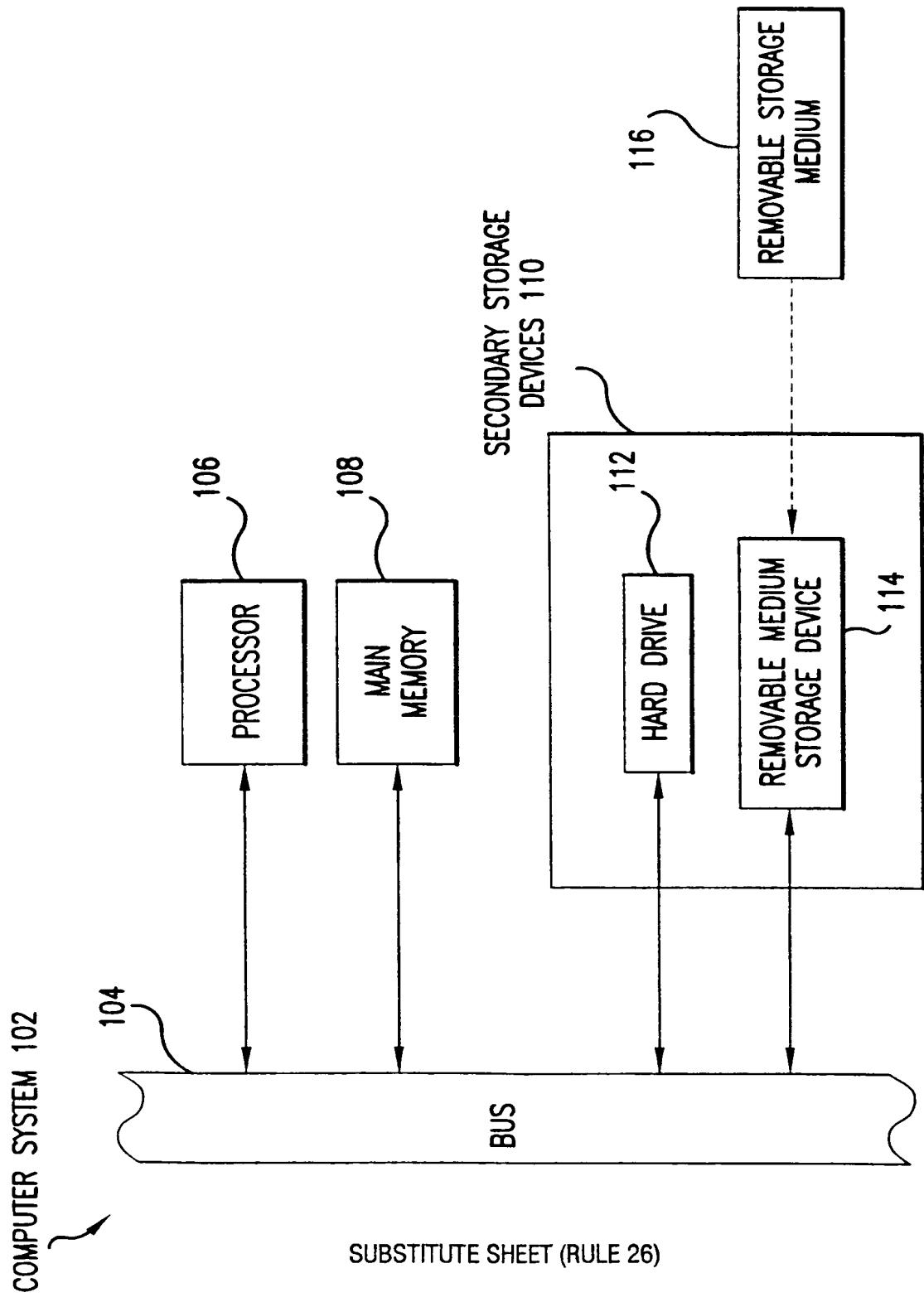


FIG.2

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 5, line 8.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

12301 Parklawn Drive  
Rockville, Maryland 20852  
United States of America

Date of deposit  
September 23, 1996

Accession Number  
97726

**C. ADDITIONAL INDICATIONS** (*leave blank if not applicable*)

This information is continued on an additional sheet

DNA plasmid PAI-1

The applicants hereby request that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

**E. SEPARATE FURNISHING OF INDICATIONS** (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications, e.g., "Accession Number of Deposit"*)

For receiving Office use only

This sheet was received with the international application

Authorized officer

For International Bureau use only

This sheet was received by the International Bureau on:

Authorized officer

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
(PCT Rule 13bis)**

**A.** The indications made below relate to the microorganism referred to in the description on page 5, line 8.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

12301 Parklawn Drive  
Rockville, Maryland 20852  
United States of America

Date of deposit  
September 23, 1996

Accession Number  
97727

**C. ADDITIONAL INDICATIONS** (*leave blank if not applicable*)

This information is continued on an additional sheet

DNA plasmid PAI-2

The applicants hereby request that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

**E. SEPARATE FURNISHING OF INDICATIONS** (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications, e.g., "Accession Number of Deposit"*)

For receiving Office use only

This sheet was received with the international application

Authorized officer

For International Bureau use only

This sheet was received by the International Bureau on:

Authorized officer

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 5, line 14.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

12301 Parklawn Drive  
Rockville, Maryland 20852  
United States of America

Date of deposit  
September 23, 1996

Accession Number  
98176

**C. ADDITIONAL INDICATIONS** (*leave blank if not applicable*)

This information is continued on an additional sheet

Escherichia coli, 596

The applicants hereby request that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

**E. SEPARATE FURNISHING OF INDICATIONS** (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications, e.g., "Accession Number of Deposit"*)

For receiving Office use only

For International Bureau use only

This sheet was received with the international application

This sheet was received by the International Bureau on:

Authorized officer

Authorized officer

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/21347

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 C12N15/11 C12N15/63 C07K16/12 G01N33/569 G06F17/30  
 G11B7/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SWENSON D.L.: "Two pathogenicity islands in <i>Escherichia coli</i> J96: cosmid cloning and sample sequencing"  <i>INFECTION AND IMMUNITY</i>,          vol. 64, no. 9, September 1996, pages 3736-3743, XP002069149          WASHINGTON US          cited in the application          see the whole document</p> <p>---</p> <p>Database EMBL, Heidelberg, DE          AC: u59875          12-NOV-1996  <i>Yersinia pestis</i> pesticin plasmid putative insertion sequence IS100          XP002069557          &amp; McDONOUGH K.A. ET AL.: <i>J. BACTERIOL.</i>,          vol. 179, 1997, pages 2081-2085,          ---</p> <p>-/-</p>	1-12, 14-17
X	<p>---</p>	1-12

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

29 June 1998

Date of mailing of the international search report

08.10.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl  
 Fax: (+31-70) 340-3016

Authorized officer

Panzica, G

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/21347

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database EMBL, Heidelberg, DE AC z32853 05-MAY-1994 Yersinia pestis insertion sequence IS100 XP002069152 see abstract & PODLACHIKOVA O. ET AL.: FEMS MICROBIOL. LETT., vol. 121, 1994, pages 269-274, ---	1-12
A	BURLAND V. ET AL.: "Analysis of the Escherichia coli genome VI: DNA sequence of the region from 92.8 through 100 minutes" NUCLEIC ACID RESEARCH, vol. 23, 1995, pages 2105-2119, XP000612159 OXFORD, GB -----	

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/21347

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see further information sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see further information sheet, subject 1.

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/21347

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

### 1. Claims: 1-20 (partially)

#### Invention 1.

An isolated nucleic acid molecule, comprising a polynucleotide having a nucleotide sequence consisting of the sequence of the ORF ID 2 (Contig Id.No.65 Start: nt1902 Stop: nt1042) and uses thereof.

### 2. Claims: 1-20 (partially)

#### Inventions 2 to 175:

same as invention 1, but according to each single ORF ID as set forth in Tables 1 through 4, starting from the second of the list (2: Contig ID 65, ORF ID 3, start: nt2096 stop: nt1821; 3: Contig ID 63, ORF ID 11, start: nt7856 stop: nt9238; ... 175: Contig ID 24, ORF ID 1, start: nt492, stop: nt4)

### 3. Claims: 21-24

#### Invention 176.

A computer readable medium having recorded thereon nucleotide sequences depicted in SEQ.ID.NOs.1 through 142 or sequences at least 99.9% identical thereto, or a complementary thereof.

Said computer readable medium selected from floppy disc, hard disc, RAM, ROM, CD-ROM.

### 4. Claim : 25

#### Invention 177.

A computer-based system for identifying fragments of uropathogenic E.coli J96 pathogenicity islands PAI IV and PAI V that are homologous to target nucleotide sequences, comprising:

- a) A data storage means comprising nucleotide sequences out of SEQ.ID.NOs:1 through 142 or a nucleotide sequence at least 99.9% identical thereto,
- b) A search means for comparing a target sequence to said nucleotide sequence of said data storage means of step a) to identify a homologous sequence,
- c) A retrieval means for obtaining said homologous sequence of step b).

